AWARD NUMBER: **W81XWH-10-1-0469**

TITLE: Genetic Evaluation for the Scoliosis Gene(s) in Patients with Neurofibromatosis 1 and Scoliosis

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REPORT DATE: October 2015

TYPE OF REPORT: **Final**

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2015	Final	1Aug2010 - 31Jul2015

4. TITLE AND SUBTITLE
"Genetic Evaluation for the Scoliosis Gene(s) in Patients with Neurofibromatosis 1 and Scoliosis."

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-1-0469

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Minnesota
Minneapolis, MN 55455

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Dystrophic or non-dystrophic forms of scoliosis are skeletal manifestations of Neurofibromatosis type 1 (NF1). Dystrophic scoliosis has a more progressive and debilitating course than non-dystrophic scoliosis, often requiring substantial surgical intervention. Experts have recommended early intervention for better outcomes but clinical tools for early detection of dystrophic scoliosis have not been developed. The first goal of this study was to develop a validated radiographic tool for differentiation of dystrophic vs. non-dystrophic scoliosis. The second goal was to determine if an existing genetic test used to predict scoliosis progression in adolescent idiopathic scoliosis could also identify dystrophic scoliosis in NF1 patients. Early detection may change the treatment paradigm. Discerning that a patient is non-dystrophic may lessen the frequency of evaluations. We found that radiographic characteristics of rib penciling, vertebral rotation, vertebral wedging, and atypical curve apex location are the most predictive of dystrophic scoliosis. If all four of these were present, there was a 96% probability that the curve was dystrophic. Genetic testing using the ScoliScore™ test showed significantly higher scores for dystrophic versus non-dystrophic scoliosis in NF1 patients. These genetic and radiographic findings will more reliably classify NF1 patients with dystrophic scoliosis.

15. SUBJECT TERMS
Neurofibromatosis Type I, Dystrophic Scoliosis, Radiographic Characteristics, Genetics

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	38	19b. TELEPHONE NUMBER *(include area code)*

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INTRODUCTION

Neurofibromatosis Type 1 (NF1) is an autosomal dominant genetic disorder occurring in 1:2500 children worldwide.[1] Scoliosis is a common skeletal disorder in pediatric patients with NF1, and may present as dystrophic (progressive) or non-dystrophic scoliosis.[2] The clinical course of non-dystrophic scoliosis is more benign than dystrophic, and similar to that of adolescent idiopathic scoliosis (AIS).[3,4] NF1 dystrophic (versus non-dystrophic) scoliosis has a poorer prognosis in terms of progression, curve severity, and impairment.[3] Dystrophic scoliosis causes distinct vertebral malformations, progresses rapidly, and curve progression may accelerate at any time, often independent of the adolescent growth spurt.[5] Patients with dystrophic scoliosis often require spine fusion surgery prior to adulthood. The magnitude of the surgery is much greater, often requiring anterior and posterior spinal fusions which have greater associated morbidity. Dystrophic scoliosis is clinically challenging for surgeons, patients, and families in terms of curve monitoring and surgical decision-making.

Radiographic criteria were developed by Durrani, et al. to assist surgeons in determining the risk of scoliosis curve progression in NF1 patients over time, a process called *dystrophic modulation*.[5] Characteristic x-ray features of dystrophic spinal deformity in NF1 patients identified by Durrani, et al. include rib penciling, vertebral rotation, vertebral wedging, anterior, lateral or posterior vertebral body scalloping, enlarged intervertebral foramina, widened interpedicular distance, and spindling of transverse processes. Durrani et al. reported that spinal curves accompanied by 3 penciled ribs or a combination of three or more dystrophic features had a high likelihood of progression.[5] In the present study, we tested a modification of the Durrani radiographic criteria with a group of expert spine surgeons.

In addition to monitoring radiographic changes over time in patients with NF1, specific genetic markers have been used to predict scoliosis curve progression in AIS.[6] However it was not known whether the same genetic markers in AIS patients are present in patients with dystrophic NF1 scoliosis.

To fill these knowledge gaps, we conducted two separate but related research studies with two distinct patient samples to inform the clinical differentiation of dystrophic from non-dystrophic scoliosis in pediatric patients with NF1. The study aims were as follows:

Aim 1. Develop and test a modified radiographic scheme to distinguish dystrophic from non-dystrophic scoliosis in Neurofibromatosis Type 1 patients that could allow for early detection and intervention.

Aim 2. Determine whether the genetic markers for curve progression in Adolescent Idiopathic Scoliosis patients are prognostic for differentiating dystrophic vs non-dystrophic scoliosis in individuals with Neurofibromatosis Type 1.

We hypothesized that:

1. Radiographic markers in isolation or combination can differentiate dystrophic (progressive) from non-dystrophic scoliosis in Neurofibromatosis Type 1 patients.

2. Genetic markers (ScoliScore™) in NF1patients will differ among those with dystrophic vs. non-dystrophic forms of scoliosis.

KEYWORDS

Neurofibromatosis Type I, Dystrophic Scoliosis, Radiographic Characteristics, Genetics

ACCOMPLISHMENTS

Major Goals of the Project

Aim 1. Develop and test a modified radiographic scheme to distinguish dystrophic from non-dystrophic scoliosis in Neurofibromatosis Type 1 patients that could allow for early detection and intervention.

Aim 2. Determine whether the genetic markers for curve progression in Adolescent Idiopathic Scoliosis patients are prognostic for differentiating dystrophic vs non-dystrophic scoliosis in individuals with Neurofibromatosis Type 1.

The Statement of Work versus the Work Performed and/or Modifications to the Statement of Work is detailed in **Table 1** below.

Table 1. Original Statement of Work versus Work Performed and/or Modifications to Statement of Work Tasks

Original Statement of Work	Work Performed and/or Modification(s) to Statement of Work Task
Task 1: Develop and validate classification scheme for scoliosis secondary to Neurofibromatosis 1.	
Preoperative radiographs of patients with dystrophic and non-dystrophic scoliosis will be evaluated. All radiographs in film format will be scanned and converted to digital format. Dr. Ledonio and Dr. Polly will collect and initially evaluate the radiographs.	De-identified sets of PA and lateral full spine radiographs for each patient were collected by The University of MN research coordinator. Dr. Polly evaluated each set of x-rays for clarity and readability.
This grading scheme will be reviewed by Drs. Crawford, Kuklo and Polly for initial face validity	• Dr. Kuklo became unavailable and did not participate in this project. • A modified list of x-ray criteria was developed by Dr. Polly and Crawford, adapted from Durrani et al. criteria.[5] • A severity grading scheme was then developed by Drs. Polly and Crawford.
A set of images will be sent to scoliosis surgeons for intra- and inter-observer reliability testing to determine generalized reliability.	• X-ray images were not sent to the surgeon panel. As preferred by the panel, the five spine surgeons met at the University of MN. Digital x-rays were displayed and reviewed simultaneously, but graded independently. The panel reviewed 122 sets of x-rays from 122 patients. The surgeon panel included Drs. Crawford, Polly, N. Larson, Carreon, and Sucato. • Expert spine surgeon panel members (*readers*) thought that the grading scheme was too complicated to use. Therefore, a grading of present or absent was used for each x-ray criterion. • Inter-observer reliability was studied; it was not possible to accomplish intra-observer reliability.

Original Statement of Work	Work Performed and/or Modification(s) to Statement of Work Task
Task 2: Identification, recruitment and informed consent acquisition of 100 NF1 patients with scoliosis from SDSG and NF support groups.	
Once identified, letters of invitation to participate in this study together with informed consent form will be sent by Dr. Polly and his staff. Barb Rogers, the research coordinator at the University of Minnesota will keep track of study participants. Dr. Christopher Moertel will be a resource for patient recruitment along with the Spinal Deformity Study Group.	• Recruitment from Spinal Deformity Study Group (SDSG) was disappointing. SDSG became dormant due to change in funding status. • NF support groups were contacted and provided some patients. • Upper age limit for patient inclusion (Aim 2) was expanded from 30 to 60 years; this change only minimally improved recruitment. • A no cost extension in study duration was obtained, largely due to recruitment challenges encountered in Aim 2.
All information on study participants will be recorded and stored in a password protected and secure computer at the University of Minnesota. Barb Rogers will maintain these records.	Barb Rogers, University of MN research coordinator, moved to another position early in the study. Her replacement was Ivana Ninkovic.
Once informed consent is obtained participants will be referred to Axial Biotech. Axial Biotech will send the participants a buccal swab kits with a self-addressed stamped envelope.	No change
Participants will be asked to swab the inside of their cheeks and to collect DNA sample and mail them back to Axial Biotech for genetic testing. They will be guided by written instructions telephone instructions and/or internet video instruction.	No changes
Task 3: Perform genetic testing on patients with NF 1 who have had clinical treatment for scoliosis at Axial Biotech with Drs. Ogilvie and Ward.	Drs. Ward and Ogilvie advised Axial Biotech on ScoliScore™ testing but did not process any samples.
Genomic DNA will be extracted from the swabs using the Autopure DNA isolation system (Gentra Systems, Minneapolis, MN).	No change
The SNPs will be genotyped using the TaqMan 5'-exonuclease SNP allelic discrimination assay by means of an ABI 7900 HT thermocycler (Applied Biosystems, Foster City, CA).	No change
Genotyping errors will be excluded by duplicate genotyping.	No change
Genotype accuracy of tested samples will be based on evaluation of control samples	No change

Original Statement of Work	Work Performed and/or Modification(s) to Statement of Work Task
of known genotypes included in each genotyping run. A concordance rate of >99% in controls will be used for to validate the accuracy of the patient genotypes. Rare missing marker genotypes for an individual patient will be given a prognostic value equal to the mean of all values observed in the original trial.	
Two cohorts will be collected, NF1 patients with dystrophic scoliosis that have been treated surgically and NF1 patients with non-dystrophic scoliosis that have been treated or reached skeletal maturity and not require surgery.	No change
Sensitivity and clinical utility will be calculated	No change
Task 4: Preparation of reports, analysis of data and preparation of manuscript	
Progress reports will be prepared and submitted as required. Dr. Ledonio together with Barb Rogers will ensure timely completion.	Progress reports were submitted by Dr. Ledonio and Ivana Ninkovic after Barb Rogers moved to another position. Per Dr. Polly's request, the final report was written and complied by Mary Forte, PhD, DC, based on draft report components from Ann Brearley, PhD (Methods & Results, Aim 1), Axial Biotech (Methods & Results, Aim 2), David W. Polly, MD, and Charles Ledonio, MD. Drs. Polly, Forte, and Moertel edited the final report. The final report submission will be approximately 1 year late.
University of Minnesota's Sponsored Project Administration will ensure appropriate submission of financial transactions.	No change
Analysis and interpretation of data will be done by Drs. Polly, Moertel, Kuklo, Crawford, Ogilvie, Ward and Ledonio assisted by the Biostatistics Design and Analysis Center.	Analysis for Aim 1 was conducted by Ann Brearley, PhD (Biostatistics Design and Analysis Center, University of MN). Analysis for Aim 2 was conducted by a CLIA-certified lab at Axial Biotech. Interpretation of data was conducted by Drs. Polly, Moertel, Crawford, Ogilvie and Ward, assisted by Ann Brearley, PhD.
Manuscript will be prepared by the investigators and collaborators.	The manuscript for Aim 1 (x-ray study) was initiated in 2014 and is still in preparation. The manuscript for Aim 2 (genetic study) is in preparation and will be submitted to an orthopedic journal in September 2016. A manuscript draft will be submitted for review by the moderator of the podium presentation session at the 51st Annual Meeting of the Scoliosis Research Society Prague, Czech Republic, September 2016.

What was accomplished under these goals?

We completed two separate but related research studies with two distinct patient samples to inform the clinical differentiation of dystrophic from non-dystrophic scoliosis in pediatric patients with NF1.

This section is organized by Study Aims. We first present the Aim 1 Methods and Results, followed by the Aim 2 Methods and Results, then a Discussion of our findings for both Aims, our Conclusions, and References.

This project was registered in Clinicaltrials.gov (NCT01776125) and approved by the following Institutional Review Boards (IRB): University of Minnesota, Cincinnati Children's Hospital Medical Center, Quorum Review IRB (for Axial Biotech) and the US Army Medical Research and Materiel Command (USAMRMC) Office of Research Protections (ORP), Human Research Protection Office (HRPO).

AIM 1

Aim 1: Develop and test a modified radiographic scheme to distinguish dystrophic from non-dystrophic scoliosis in Neurofibromatosis Type 1 patients that could allow for early detection and intervention.

Overview

Our recruitment goal for Aim 1 was 150 patients (150 sets of PA and lateral full spine radiographs); our actual enrollment was 122 patients (81.3% of goal). Posterior-to-anterior (PA) and lateral full spine radiographs from 122 patients with NF1 were included (2 x-rays per patient). Each set of radiographs was reviewed and classified five times, once by each surgeon reader. The total number of ratings by the 5-surgeon panel of independent readers was 610 ratings [610 = 5 readers*(122 PA & lateral x-ray sets)].

Methods

We included patients diagnosed with Neurofibromatosis Type 1 (NIH criteria[7] and/or evidence of NF1 mutation on molecular testing from a reputable laboratory), had clinical treatment of scoliosis (either spinal fusion or patient had reached skeletal maturity), had clear and readable preoperative radiographs of the spine, and were age 3 to 30 years at the time of study x-rays. We excluded NF1 patients with paraspinal tumors causing scoliosis. We used de-identified radiographs from multiple institutions; therefore, this phase was exempt from IRB review at the University of Minnesota (Category 4 Exemption).

A team of NF1 scoliosis experts was recruited; these included pediatric orthopedic surgeons and spine surgeons (total of 5 surgeons). De-identified radiographic images from NF1 patients with dystrophic and non-dystrophic scoliosis were collected from multiple centers. The reference standard for a dystrophic scoliosis diagnosis was the diagnosis from the patient's treating (*home*) institution (dystrophic or non-dystrophic NF1 scoliosis). The home institution's diagnosis was based upon more information than radiographic findings alone; typically, MRI and/or CT imaging of the spine were included.

All radiographs in film format were scanned and converted to digital format at the University of MN. A total of 123 sets of PA and lateral full spine radiographs (123 patients =252 images) were collected. Dr. Polly initially evaluated all x-ray images for clarity and readability. One case was

excluded for poor image quality, leaving 122 sets of PA and lateral x-rays from 122 patients with NF1 and scoliosis for Aim 1.

Drs. Crawford and Polly modified the list of radiographic criteria from Durrani et al.[5]. Specifically, they combined anterior, lateral, and posterior vertebral body scalloping into one criterion; added a criterion for *short, sharp angular curve*; and deleted the enlarged IVF criterion (**Table 2**). Dr. Polly and Crawford developed definitions for each of 8 final radiographic criteria; some definitions were adapted from the Durrani et al. definitions[5](**Table 2**).

Table 2. Modified Radiographic Criteria for Identification of Dystrophic Scoliosis*

X-ray characteristic	Definition
Rib penciling	Rib width smaller than narrowest portion of second rib
Vertebral rotation	Rotation as compared to level above and below
Vertebral scalloping	Anterior, lateral and/or posterior scalloping compared to level above and below
Vertebral wedging	10 degrees of wedging across a single segment
Short, sharp angular curve	Cobb angle 20-30° or more across two segments
Spindling of transverse processes	<50% expected diameter compared with either the contralateral normal side or uninvolved vertebra above or below in same anatomic segment of spine
Widened interpedicular distance	Widened compared to level above and below
Atypical curve location	High thoracic, low lumbar or cervical curve

*Modification of criteria of Durrani, et al.[5]

Five expert spine surgeons (Dr. Polly, Crawford, Sucato, Larson, Carreon) met collectively at the University of Minnesota. Drs. Polly and Crawford explained their modified radiographic criteria (**Table 2**) to the group. A modified Delphi process was used to clarify and agree upon the definitions used for defining the presence or absence of each radiographic parameter.

We projected 122 sets of radiographic images to the panelists who independently graded each criterion as present or absent. Since there are no established criteria for aggregate scoring of these modified criteria [5], panelists provided their global impression of the presence or absence of dystrophic modulation.

Data from the 5-surgon panel were entered into an Excel database and sent to a statistician for analysis in SAS 9.2 (Cary, NC, USA) and R.

Statistical analysis

Our goal was to estimate the reliability of inter-observer evaluations of the presence or absence of dystrophic scoliosis. Given the reference standard diagnosis from the patient's home institution, we assessed global rating accuracy. We then performed univariate and multivariate radiographic factor analysis. Lastly, we looked at estimating the sensitivity and specificity of radiography-based diagnosis of dystrophic scoliosis relative to the 'gold standard' of a definitive clinical diagnosis (pre-enrollment) and estimated inter-observer reliability across the five panelists.

Inter-observer Reliability

The dichotomous outcome of interest was whether a patient's radiograph indicated dystrophic scoliosis (yes/no). We quantified the inter-observer agreement (Kappa statistic[8,9]) for the presence or absence of each radiographic characteristic associated with dystrophic NF1 across 5

surgeon readers. We used the *%MAGREE* macro in SAS to calculate the Kappa statistics based on the methodology of Fleiss[8], and double-checked our results using the *kappam.fleiss* function in R.

Predictive Ability – Sensitivity and Specificity

We determined how well each of the eight radiographic characteristics alone predicted dystrophic scoliosis using standard diagnostic test criteria of sensitivity and specificity. We assessed which combinations of characteristics were associated with dystrophic scoliosis using multiple logistic regression. The binary outcome was dystrophic NF1 scoliosis (yes/no), with the 8 radiographic characteristics tested as binary predictors.

The sample size for assessing the sensitivity and specificity of x-ray predictors for Aim 1 was estimated assuming that the test sensitivity and specificity will both be 90% and that we would like the 95% exact binomial confidence intervals for each to be (80%, 98%). This required a sample size for these criteria was 75 dystrophic plus 75 non-dystrophic sets of patient radiographs (150 sets).

Results

Sample

We included x-rays from 122 patients with NF1 and scoliosis; 68.0% had been diagnosed with dystrophic scoliosis by their treating (*home*) institution prior to study enrollment. Demographic features of our patient sample were indeterminable because radiographs were de-identified.

Our recruitment goal for Aim 1 was 150 patients (150 sets = 300 radiographs). However, several institutions that initially offered to provide de-identified radiographs were precluded from submitting x-rays by their institutional review boards (most notably, Boston Children's Hospital).

Proportion of Patients with Dystrophic Scoliosis

Collectively, 363 (59.5%) out of 610 sets of radiographs from 122 patients were classified by 5 surgeons as having dystrophic scoliosis. Of the 122 patients, 83 (68.0%) had an established diagnosis of dystrophic scoliosis from their treating institution at the time of study enrollment. Based on radiographs alone, all of the surgeon experts underestimated the proportion of patients with dystrophic scoliosis. For a given surgeon, the proportion deemed dystrophic ranged from 45.1% to 67.2%, and these between-surgeon differences are statistically significant (p-value=0.006, **Table 3**).

Table 3. Proportion of 122 Patients Classified As Having Dystrophic Scoliosis by Individual Surgeon Panelists Based on PA and Lateral Full Spine Radiographs*

Surgeon Reader	Dystrophic n (%)	Number of x-ray sets read	p value**
1	75 (61.5)	122	
2	77 (63.1)	122	
3	82 (67.2)	122	0.006
4	74 (60.7)	122	
5	55 (45.1)	122	
Total	**363 (59.5%)**	**610**	

*Using a modification of Durrani et al.[5] **Pearson's chi-square. PA=posterior to anterior

Accuracy of Surgeon Ratings (Sensitivity and Specificity)

We compared the home institution's diagnosis of dystrophic scoliosis to surgeons' diagnosis of the 610 x-ray sets (**Table 4)**. Among 83 patients with an enrollment diagnosis of dystrophic scoliosis, surgeon experts were correct 74.7% of the time based only on a single set of PA & lateral full spine radiographs (i.e. the overall sensitivity was 75%). Among patients with an enrollment diagnosis of non-dystrophic scoliosis from their home institution, surgeon raters were correct 72.8% of the time (i.e. the overall specificity was 73%).

Table 4. Overall Proportion of Patients Classified As Having Dystrophic Scoliosis by 5-Surgeon Panel Based on Radiographs

	Actual diagnosis		
Surgeons' classification	**Dystrophic**	**Non-dystrophic**	**Total**
Dystrophic scoliosis	310 (74.7%)	53 (27.2%)	363
Non-dystrophic scoliosis	105 (25.3%)	142 (72.8%)	247
Total x-ray sets read:	**415**	**195**	**610***

*610 x-ray sets read: 5 surgeons read PA& lateral full spine x-ray sets from 122 patients

Categorical interpretation of the Kappa statistics in subsequent results are based on the commonly-used but arbitrary categories suggested by Landis and Koch[10] (**Table 5**).

Table 5. Strength of inter-rater agreement categories for the Kappa statistic proposed by Landis and Koch[10]

Kappa statistic	Strength of agreement
0.81 – 1.00	Almost perfect
0.61 – 0.80	Substantial
0.41 – 0.60	Moderate
0.21 – 0.40	Fair
0.00 – 0.20	Slight
<0.00	Poor

The sensitivity, specificity and agreement with the true diagnosis per reader are shown in **Table 6**. The overall agreement between the enrollment diagnosis from each patient's home institution compared with surgeons' diagnosis, assessed using the kappa statistic, is 0.44 or *moderate*.[10] Individual reader agreement with the true diagnosis is *moderate* for each expert surgeon.

Table 6. Sensitivity, specificity and Kappa statistics of rated versus actual dystrophic diagnosis for each surgeon reader in NF1 patients with scoliosis

Surgeon reader	**Sensitivity** %	**Specificity** %	**Agreement with true diagnosis** (kappa)
1	77.1	71.8	0.46
2	77.1	66.7	0.42
3	83.1	66.7	0.49
4	74.7	69.2	0.41
5	61.5	89.7	0.43
Overall (average)	**74.7**	**72.8**	**0.44**

Inter-observer Reliability

The Fleiss' Kappa values for the overall dystrophic diagnosis and the eight individual radiographic characteristics from 122 sets of x-rays of NF1 patients rated by 5 surgeons are shown in **Table 7.** The degree of agreement between 5 expert surgeons ranged from the lower end of *substantial* for the overall diagnosis and vertebral wedging to *slight* for vertebral scalloping and widened interpedicular distance.[10]

Table 7: Inter-rater Agreement for Individual Radiographic Characteristics Associated With Dystrophic Scoliosis as Assessed by Five-Surgeon Panel

Characteristic	Kappa statistic[8]	Strength of agreement[10]
Dystrophic diagnosis	**0.61**	**substantial**
Vertebral wedging	0.62	substantial
Vertebral rotation	0.59	moderate
Sharp angular curve	0.60	moderate
Rib penciling	0.41	moderate
Vertebral scalloping (any)	0.14	slight
Widened interpedicular distance	0.18	slight
Atypical curve location	0.28	fair
Spindling of transverse processes	0.42	moderate

The proportion of x-ray sets that were rated as having specific dystrophic radiographic characteristics are shown in **Appendix A**. The characteristics most frequently observed in x-rays deemed dystrophic were vertebral wedging, vertebral rotation and sharp angular curve.

The frequency at which specific dystrophic characteristics were observed in radiographs of NF1 patients with dystrophic scoliosis are shown in **Appendix B**. The association between each characteristic and true dystrophic diagnosis is highly significant (chi-square test, p-value < 0.0001) for seven of the eight characteristics, and significant for the eighth (spindling, p-value = 0.0011).

Dystrophic classification

Inter-observer variability was further investigated by counting the number of times a given characteristic was said to be present by the five readers (**Appendix C**). Of the 83 sets of x-rays from patients diagnosed with dystrophic scoliosis prior to enrollment, 42 (50.6%) were correctly classified as dystrophic by all five surgeon readers; only 8 patients (9.6%) were incorrectly classified as *non*-dystrophic by all five x-ray readers. There was a degree of disagreement for the remaining 33 (39.8%) sets of x-rays from dystrophic patients. Of the 39 non-dystrophic x-ray sets, 22 (56.4%) were classified correctly by all five readers, four (10.3%) were classified incorrectly by all five readers, and there was some disagreement about the remaining 13 (33.3%).

Logistic regression

Logistic regression modeled the association between radiographic characteristics and dystrophic scoliosis (yes/no). Our goal was to determine which combination of radiographic characteristics was best able to predict a true dystrophic diagnosis for the N = 610 readings. The log odds of an x-ray being truly dystrophic (versus non-dystrophic) were initially modeled as a function of the eight modified radiographic characteristics. No higher order or interaction terms were considered or used in the analysis.

The final model included 4 x-ray characteristics: vertebral rotation, vertebral wedging, rib penciling, and atypical curve location. Forward, backward and stepwise variable selection did not change model inclusion. We eliminated four characteristics (vertebral scalloping, widened interpedicular distance, sharp angular curve, and spindling of transverse processes) because preliminary models (backward elimination) indicated that these factors were not significantly associated with dystrophic scoliosis.

All 4 characteristics (vertebral rotation, vertebral wedging, rib penciling, and atypical curve location) were significantly associated with a dystrophic scoliosis diagnosis at enrollment (**Table 8**). The odds of having dystrophic NF1 scoliosis were 3.00 times higher if a surgeon identified an atypical scoliosis (curve) location (vs. not), 2.98 times higher if the reader saw vertebral rotation (vs. not) and 2.43 times higher when the reader saw rib penciling (vs. not).

Table 8: Logistic Regression: Odds ratios of having dystrophic NF1 scoliosis (vs. non-dystrophic) by specific radiographic features

X-ray Characteristic	Odds ratio	95% confidence limit	P value
Rib penciling	2.43	1.51, 3.92	0.0003
Vertebral rotation	2.98	1.85, 4.79	<0.0001
Vertebral wedging	2.37	1.47, 3.82	0.0004
Atypical curve location	3.00	1.57, 5.72	0.0009

The model-predicted probability of having dystrophic scoliosis (blue dots) and the actual probability of having dystrophic scoliosis (red squares) are given in **Figure 1** below, as a function of a 5-character summary variable pattern (**Table 9**) created as follows:

- **First digit** indicates the count of four characteristics (vertebral rotation, vertebral wedging, rib penciling, and atypical curve location) that were observed in a given reading.
- **Remaining four characters** indicate the presence (Y=yes) or absence (N=no) of four characteristics (vertebral rotation, vertebral wedging, rib penciling, and atypical curve location), in that order. For example, the pattern for the last 4 characters would be NNNN if all four characteristics were not identified by the reader and YNNN if the reader observed only vertebral rotation.
- **Example:** The pattern would be 2YNYN if a reader saw vertebral rotation and rib penciling.

Since each set of x-rays was read five times by surgeons, and the five surgeons did not always agree, a given x-ray set may contribute to as many as five different patterns.

Table 9. Model-predicted probabilities of Dystrophic NF1 Scoliosis Based on a Summary Variable of Four Radiographic Characteristics* as the predictor

Number of factors noted (out of 4)	Vertebral rotation	Vertebral wedging	Rib penciling	Atypical curve location	Predicted probability of dystrophic	Actual % dystrophic
0	N	N	N	N	31.53	34.19
1	N	N	N	Y	57.98	66.67
1	N	N	Y	N	52.83	52.94
1	N	Y	N	N	52.16	50.00
1	Y	N	N	N	57.82	43.33
2	N	N	Y	Y	77.04	50.00
2	N	Y	N	Y	76.56	25.00

Number of factors noted (out of 4)	Vertebral rotation	Vertebral wedging	Rib penciling	Atypical curve location	Predicted probability of dystrophic	Actual % dystrophic
2	N	Y	Y	N	72.62	64.29
2	Y	N	N	Y	80.42	75.00
2	Y	N	Y	N	76.93	80.00
2	Y	Y	N	N	76.45	79.17
3	N	Y	Y	Y	88.82	85.71
3	Y	N	Y	Y	90.90	100.00
3	Y	Y	N	Y	90.68	95.86
3	Y	Y	Y	N	88.76	88.49
4	Y	Y	Y	Y	95.95	98.46

*vertebral rotation, vertebral wedging, rib penciling, and atypical curve location. Y=yes, reader identified feature on x-ray; N= no, reader did not identify feature

The model predictions (**Table 9** and **Figure 1**) are reasonably close to the actual values (**Table 9**, last column). The model predicts that the probability of a person having dystrophic scoliosis based on one set of x-rays is about 31% if the reader saw none of the four characteristics (vertebral rotation, vertebral wedging, rib penciling, and atypical curve location). The probability of dystrophic scoliosis rises to approximately 52-58% if the reader saw one of four characteristics, 72-80% if two characteristics were identified, 88-91% if three were noted, and 96% if a surgeon identified all four radiographic features in a single set of full-spine x-rays.

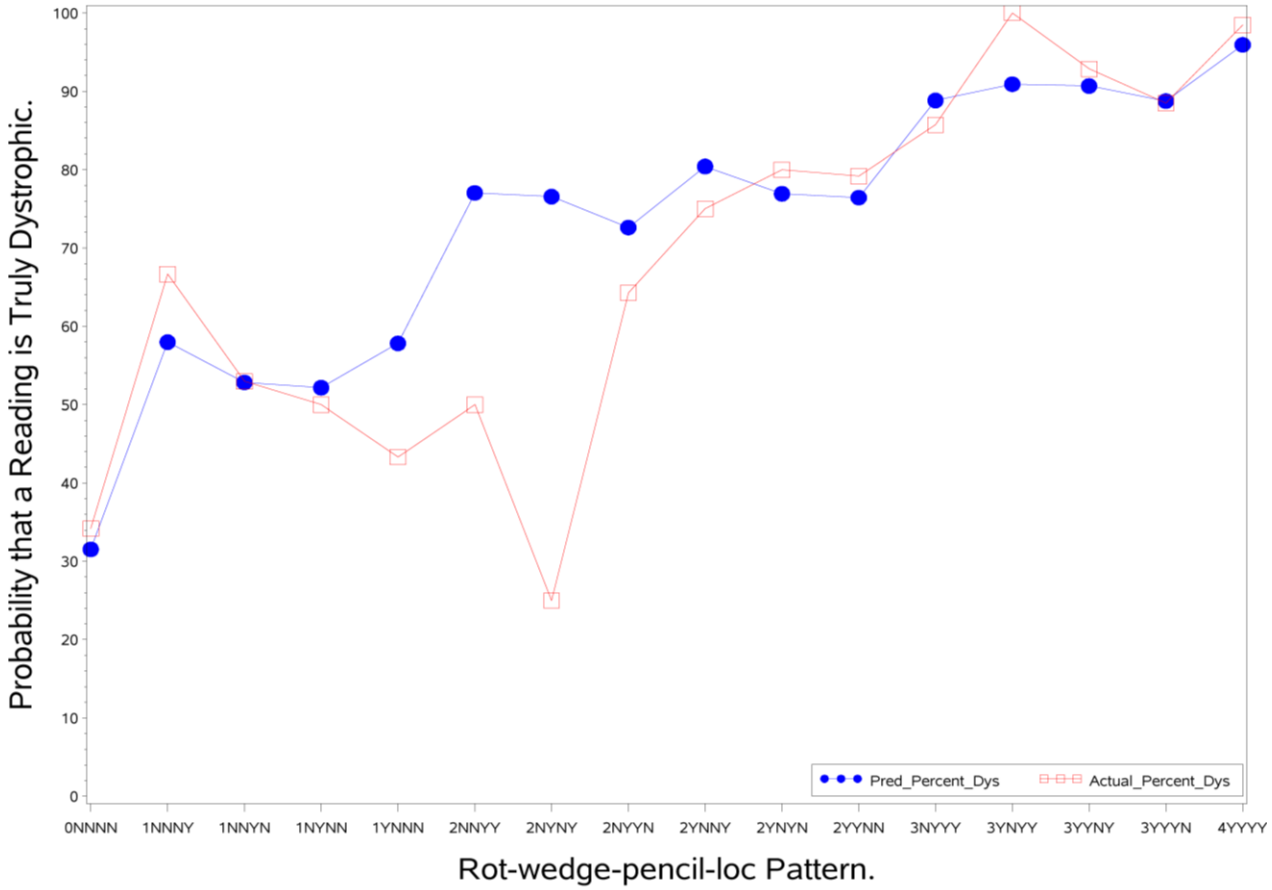


Figure 1: Graph of predicted probabilities of dystrophic scoliosis from logistic regression, based on a 5-character summary pattern of radiographic features: vertebral rotation and wedging, rib penciling, and atypical curve location

AIM 2

The goals of Aim 2 were to perform genetic testing on patients with NF1 who had clinical treatment for scoliosis and correlate those findings with the radiographic information that distinguished dystrophic from non-dystrophic scoliosis in Aim 1. Aim 2 recruitment was separate from Aim 1.

Methods

Sample selection and recruitment

Identification and recruitment of subjects came via the members of the Spinal Deformity Study Group and the Children's Tumor Foundation (http://www.ctf.org) who hosts the Neurofibromatosis Clinic Network. We included patients with a diagnosis of Neurofibromatosis Type 1[7] and scoliosis who had either reached skeletal maturity or had required surgical treatment/ spinal fusion for scoliosis; had proper preoperative radiographs of the spine; and were age 3 to 60 years old. We excluded individuals with paraspinal tumors causing scoliosis.

Patient Recruitment and Informed Consent

Patient recruitment processes were approved by the University of Minnesota IRB prior to recruitment initiation.

Candidates were identified by spine surgeons who are members of the Spinal Deformity Study Group. These are individuals who have been clinically diagnosed with NF 1 according to NIH criteria[7] and had undergone spinal fusion for scoliosis (either dystrophic or non-dystrophic).

Patients were contacted by the research team from the University of Minnesota, and asked for their interest to participate. If they agreed, parental consent and assent forms were sent to the patient and their parents. The research coordinator at the University of Minnesota kept track of study participants. Dr. Christopher Moertel was a resource for patient recruitment, along with the Spinal Deformity Study Group, and Children's Tumor Foundation. Also included were patients from Cincinnati Children's Hospital with Dr. Alvin Crawford as the site PI.

Once consent was obtained, the research coordinator from the University of Minnesota collected digital radiographs of their scoliosis. This information was stored on a secure password-protected University of Minnesota server. In the event that digital radiographs were not available, radiographic plain films were requested, scanned and digitized.

Once enrolled, the patients received a cheek swab collection kit with instructions via mail from either a genetic testing lab or, if the patient lived locally, from the University of Minnesota. The patient or parent collected the sample per the provided instructions, and returned the sample back to the genetic testing laboratory in a prepaid envelope. Genetic testing was performed, and the results were recorded.

Due to disappointing enrollment using our planned approach, additional patient recruitment efforts were proposed, IRB-approved and implemented; this included the use of the University of Utah's DNA bio-bank which contained blood samples and de-identified clinical records and radiographs for patients diagnosed with NF1. Those radiographs were screened and reviewed by Dr. Polly to determine if the patient met the inclusion criteria. Qualified samples were then sent for genetic testing.

Lab Testing and Genotyping

This section provides a general overview of the methods used for DNA testing and analysis, followed by specific details of the process we used.

Genetic testing was conducted at Axial Biotech and, after its closure in March 2013, at Affiliated Genetics. DNA collection and genotyping of the sample cohorts identified 53 single-nucleotide polymorphism (SNP) markers associated with scoliosis progression to a spinal curve that necessitated surgical intervention among AIS patients previously identified by Ward, et al.[6]. The results of the SNP genetic marker analysis are represented as a numerical summary score, with higher values associated with a higher risk of curve progression.[6]

Specifically, cheek swabs were collected in a DNA Genotek (Ottawa, Canada), Oragene OG-300 sample collection kit. DNA samples were then extracted from the cheek swabs using MagNA Pure Compact magnetic bead extraction protocols (Roche Applied Sciences, Indianapolis, IN). Genotypes were determined using 53 Taqman™ assays (Applied Biosystems, Inc., Foster City, CA) designed to detect each SNP. The Taqman™ assay is an allele discrimination assay using polymerase chain reaction (PCR) amplification and a pair of fluorescent dye detectors that target each SNP. One fluorescent dye is attached to the detector that is a perfect match to the first allele (e.g. an "A" nucleotide) and a different fluorescent dye is

attached to the detector that is a perfect match to the second allele (e.g. a "C" nucleotide). During PCR, the polymerase releases the fluorescent probe into solution where it is detected using endpoint analysis in an Applied Biosystems 7900HT Real-Time instrument. Genotypes were then determined using Applied Biosystems automated Taqman™ genotyping software, SDS v2.3. After genotypes were identified, the risk of curve progression score was determined for each patient using a logistic regression algorithm established during the discovery and validation phases of the original research.[6] All samples and scores were tracked in a Laboratory Information Management System. Testing was done in CLIA/CAP accredited laboratory.

A ScoliScore™ Adolescent Idiopathic Scoliosis Prognostic Test (Transgenomic Inc., Omaha NE) was generated for each sample. The scoring algorithm uses a weighted average of 53 single-nucleotide polymorphism (SNP) markers to generate a summary score.[6]

Analysis

Aim 2 evaluated the clinical utility of a set of genetic markers in NF1 patients who had clinical treatment for scoliosis. These genetic markers were previously validated as markers associated with the progression of spinal curves (> 40 degree Cobb angle) in adolescent idiopathic scoliosis patients. This study attempted to confirm, in NF1 surgical patients with non-dystrophic scoliosis, the 85% sensitivity observed in surgical adolescent scoliosis patients.

Collaborators who performed the DNA tests were blinded to the type of scoliosis. ScoliScores™ were compared between the types of scoliosis using Mann-Whitney test and graphed on a box plot**.**

We used unadjusted logistic regression to predict the probability of having dystrophic scoliosis (outcome) in NF1 patients based on the ScoliScore™ genetic test as the sole predictor variable. The discriminating power of the model was accessed using area under the curve (AUC) characteristics.

Sample Size Determination

Two cohorts were collected, NF1 patients with dystrophic scoliosis that had been treated clinically and NF1 patients with non-dystrophic scoliosis that had been treated clinically. A sample size of at least 100 patients is required to evaluate the sensitivity (lower 95% CI = 0.70 to 0.75; calculations not shown). In anticipation of enrollment drop-outs we are approved to recruit 140 subjects to meet sample size requirement of 100 patients.

Results

Patient Recruitment

Our Aim 2 recruitment goal was 100 patients. We sent 1200 letters to patients diagnosed with NF type 1. Of these, 54 qualified for the study; 10 were subsequently excluded when we determined that they did not meet inclusion criteria. To enhance enrollment, we obtained IRB approval and subsequently utilized several different social media venues by advertising our study on Children's Tumor Foundation and The Littlest Tumor Foundation Midwest Society. The study was also posted on ClinicalTrials.gov. Expanding our efforts in this manner allowed us to recruit 11 more individuals (55 to this point). Additionally, we used our collaboration with the University of Utah to enroll 19 additional individuals from their genetic biobank; their genetic information was already at the University of Utah and cheek-swabs were not required in this

subset. The total number of patients included from these efforts was 74. Of those, 17 (23%) were excluded for the following reasons: did not return consent form (n=8), did not return buccal swabs (5), x-rays lacked scoliosis (2), and lacked credible x-rays/records (2).

A total of 57 patients with NF1 and with clinically and radiographically confirmed scoliosis completed all components of Aim 2. Based on radiographic characteristics and medical record review, there were 29 patients with dystrophic scoliosis and 28 patients with non-dystrophic scoliosis. The average patient age was 22 years.

Genetic Testing

The results of the Single-Nucleotide Polymorphism (SNP) marker analysis are represented as a numerical score (ScoliScore™, **Appendix D**). Higher scores are associated with a higher risk of curve progression in AIS.[6]

Median ScoliScores™ in NF1 patients were significantly higher among dystrophic than non-dystrophic individuals (35 vs. 15 respectively, Mann-Whitney test p=0.039, **Table 10** and **Figure 2**). The box plots (**Figure 2**) show asymmetric distributions for these genetic test results in both groups, with greater variation in ScoliScores™ among patients with dystrophic vs. non-dystrophic scoliosis (interquartile range 46 vs. 33 respectively); the non-dystrophic group has one high outlier).

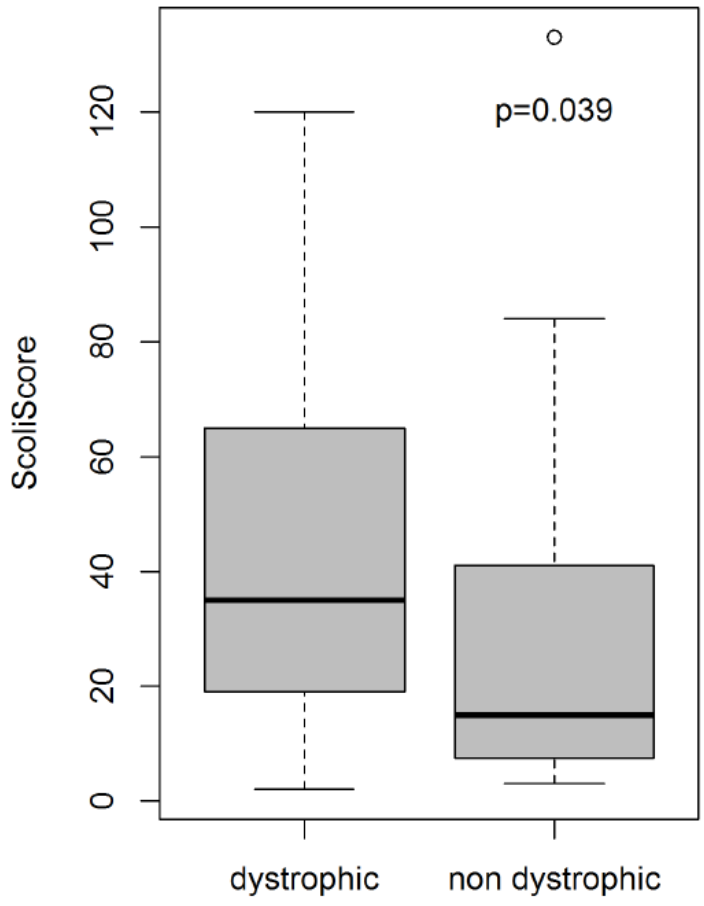


Figure 2. Box Plots of Genetic Test Scores (ScoliScore™) in Patients with Dystrophic and Non-dystrophic Neurofibromatosis Type I Scoliosis

Table 10. Genetic Test (ScoliScore™) Results for 57 NF1 patients with scoliosis, based on diagnosis from their treating institution*

Diagnosis	N (%)	Median	Mean	25th percentile	75th percentile	Inter-quartile range	Range
Dystrophic	29 (50.9)	35	44	19	65	46	118
Non-dystrophic	28 (49.1)	15	29	8	41	33	130

*Supporting data for box plots, Figure 2

Logistic regression

Risk scores generated from regression predicted dystrophic scoliosis in NF1 patients; the discriminating power of the model was assessed as the area under the curve (AUC=0.66). We predicted the probability of dystrophic scoliosis in 57 NF1 patients for ScoliScore™ values ranging from 1 to 200, along with the 95% confidence intervals estimated from the model as shown in **Appendix E**. The confidence limits around these estimates are wide (**Appendix E**). Nonetheless, the model estimated that for a NF1 patient with a ScoliScore™ of 123 or greater, the likelihood of dystrophic scoliosis is at least 80% (**Figure 3**).

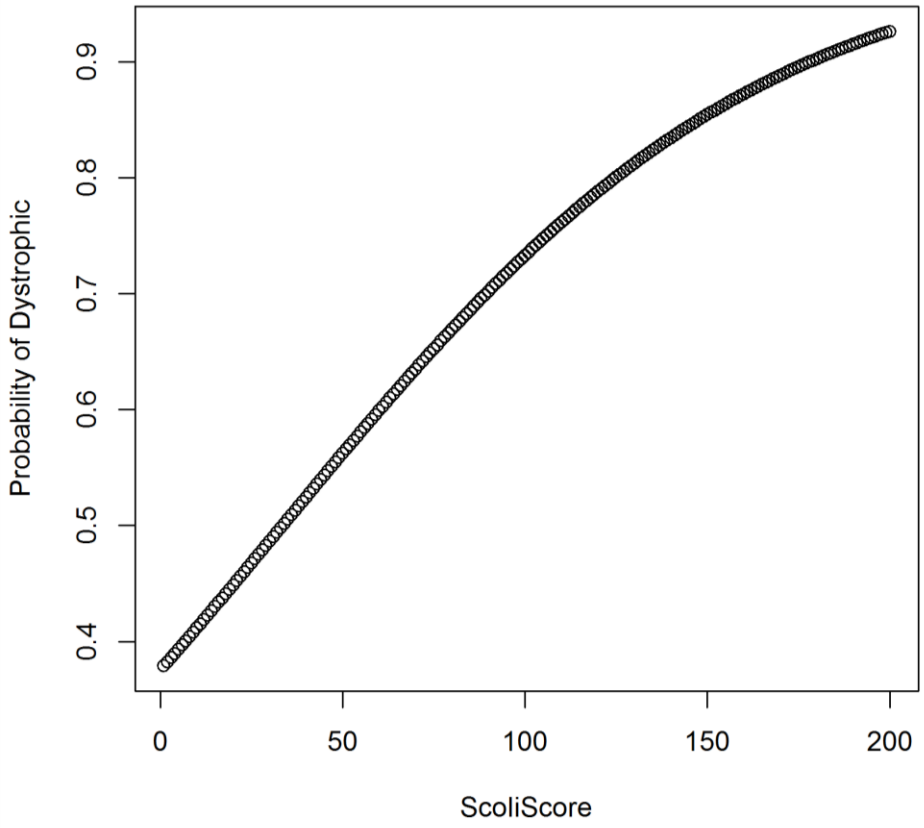


Figure 3: **Probability of Dystrophic Scoliosis Based on the ScoliScore™ Genetic Test**

Discussion

We conducted two studies with unique patient samples to inform the clinical differentiation of dystrophic from non-dystrophic scoliosis in pediatric patients with Neurofibromatosis Type I.

In Aim 1, we modified and validated radiographic criteria to differentiate dystrophic from non-dystrophic scoliosis in patients with Neurofibromatosis Type 1. Collectively, the eight-feature scoring system has moderate reliability in classifying dystrophic from non-dystrophic scoliosis using a single set of plain radiographs in the hands of expert spine surgeons. The characteristics most often observed in x-rays labeled by surgeons as dystrophic were vertebral wedging, vertebral rotation and a sharp, angular curve. The greater the count of x-ray features present per patient, the more likely surgeons were to classify the scoliosis as dystrophic. Similarly, the regression model predicted a 96% probability of a person having dystrophic scoliosis if a surgeon identified all four radiographic features of rib penciling, vertebral rotation, vertebral wedging, and atypical curve apex location, in a single set of full-spine x-rays.

Our goal in Aim 1 was to assess the utility of plain radiographs to inform early differentiation of dystrophic from non-dystrophic curves in Neurofibromatosis Type I patients who were developing scoliosis. A single set of full spine radiographs provided a small piece of diagnostic information used to determine dystrophic modulation clinically. The pre-enrollment diagnosis of dystrophic scoliosis from each patient's treating institution was also based on CT and/or MRI imaging results, and longitudinal radiographic data that were not available to the surgeon panel in Aim 1. We did not test a more realistic clinical scenario of assessing serial spinal x-rays over time, compare the reliability of the modified vs. original criteria among expert surgeons, or assess the reliability of the modified criteria using a mixed panel of spine and/or general practice orthopaedic surgeons. Our results suggest that a single set of plain radiographs provide clinically useful information that continues to aid in the diagnosis of dystrophic scoliosis. If specific radiograph changes are present, then the probability that the curve is dystrophic can be calculated. However when the radiographic changes are *not* present, we cannot comment on the probability that the curve will become dystrophic. This limitation is the reason for the search for a genetic test that could potentially provide for earlier discernment that a curve will become dystrophic.

In Aim 2, the multiple SNP analysis obtained from ScoliScore™ genetic testing suggests that there are some differences in the genetic basis between dystrophic and non-dystrophic scoliosis patients. However, we suggest caution in over-interpreting these preliminary results from our underpowered study; our results should be confirmed in a larger, sufficiently powered study. Additionally, our analysis included partial, not full DNA sequencing. These Aim 2 results present an intriguing hypothesis that dystrophic scoliosis may be pre-determined based on a patient's genetic composition. Validation of these findings in a larger sample, and subsequent testing of prediction models that incorporate additional demographic, clinical and timing information, may provide better contextual information regarding the benefits and utility of early genetic testing in young NF1 patients who are at risk for dystrophic scoliosis development.

We encountered a number of barriers to patient recruitment during both studies, but particularly for the genetic study; most barriers were previously identified in Table 1 and under *Patient Recruitment* for Aim 2. Barriers to recruitment included the dissolution of the Spine Deformity Study Group whose efforts helped initial enrollment. Barriers unique to the genetic study included stringent inclusion criteria (must have had credible & retrievable x-rays and provide a genetic sample), plus incomplete return of the buccal swabs used for genetic testing. A subsequent grant from the Department of Defense has established a NF1 consortium; this consortium will most likely allow for higher patient recruitment and greater access to clinical and radiographic data for future, related studies.

If genetic testing is highly predictive in one or more sufficiently powered clinical studies, it would alter the clinical pattern of monitoring NF1 patients with scoliosis; non-dystrophic individuals less and those with a dystrophic genetic predisposition, more. Earlier diagnosis of dystrophic scoliosis could inform clinical decision-making regarding early surgical intervention, allowing for better surgical correction of lower magnitude spinal curves, and potentially reducing surgical complications peri- and post-operatively in these generally high-risk children.

Conclusions

We conducted two related studies with distinct patient samples to inform the clinical differentiation of dystrophic from non-dystrophic scoliosis in pediatric patients with NF1. Radiographic findings from a single set of full spine x-rays provide diagnostic information that is moderately reliable across spine surgeons, and show continued utility as a diagnostic tool for dystrophic scoliosis in patients with NF1. The unique finding of this project is the positive association between high genetic test scores (ScoliScore™) and dystrophic scoliosis in our small NF1 cohort. These intriguing preliminary results merit further investigation. If substantiated in a larger sample, genetic testing, alone or in combination with clinical and related information, could allow for earlier diagnosis and intervention for pediatric patients with dystrophic scoliosis that could potentially improve outcomes.

References

1. Evans DG, Howard E, Giblin C, et al. Birth incidence and prevalence of tumor-prone syndromes: estimates from a UK family genetic register service. *Am J Med Genet A.* 2010;152A(2):327-332.
2. Akbarnia BA, Gabriel KR, Beckman E, Chalk D. Prevalence of scoliosis in neurofibromatosis. *Spine (Phila Pa 1976).* 1992;17(8 Suppl):S244-248.
3. Crawford AH, Herrera-Soto J. Scoliosis associated with neurofibromatosis. *The Orthopedic clinics of North America.* 2007;38(4):553-562, vii.
4. Weinstein SL, Zavala DC, Ponseti IV. Idiopathic scoliosis: long-term follow-up and prognosis in untreated patients. *The Journal of bone and joint surgery. American volume.* 1981;63(5):702-712.
5. Durrani AA, Crawford AH, Chouhdry SN, Saifuddin A, Morley TR. Modulation of spinal deformities in patients with neurofibromatosis type 1. *Spine (Phila Pa 1976).* 2000;25(1):69-75.
6. Ward K, Ogilvie JW, Singleton MV, Chettier R, Engler G, Nelson LM. Validation of DNA-based prognostic testing to predict spinal curve progression in adolescent idiopathic scoliosis. *Spine (Phila Pa 1976).* 2010;35(25):E1455-1464.
7. National Institutes of Health. Consensus Development Conference Statement: Neurofibromatosis. Bethesda, MD, July 13-15, 1987. *Neurofibromatosis.* 1988;1(3):172-178.
8. Fleiss JL, Levin B, Paik MC. *Statistical Methods for Rates and Proportions.* Third ed: John Wiley & Sons, Inc., Hoboken, NJ; 2003.
9. Cohen J. A coefficient of agreement for nominal scales. *Educ. Psychol. Meas.* 1960;20:37-46.
10. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics.* 1977;33(1):159-174.

What opportunities for training & professional development has the project provided?
Nothing to report

How were the results disseminated to communities of interest?
See Products below.

What do you plan to do during the next reporting period to accomplish the goals?
Nothing to report

IMPACT

What was the impact on the development of the principal discipline(s) of the project?
Nothing to report

What was the impact on other disciplines?
Nothing to report

What was the impact on technology transfer?
Nothing to report

What was the impact on society beyond science and technology?
Nothing to report

CHANGES/PROBLEMS

Nothing to report for this period. Modifications to study approaches are identified in Table 1; barriers to recruitment are detailed in the Discussion.

PRODUCTS

Presentations

Moertel CL, Polly DW, Ledonio CGT. *Radiographic Assessment Reliability of Dystrophic Modulation in NF1 Patients with Scoliosis*. Children's Tumor Foundation, University of Minnesota Symposium; Minneapolis, Minnesota, May 16, 2012.

Polly DW, Ledonio CGT, Moertel CL. *Neurofibromatosis Type I and Scoliosis: A Multicenter Study to Determine Radiographic Predictors of Dystrophic Scoliosis.* International Congress on Early Onset Scoliosis and Growing Spine (ICEOS). Dublin, Ireland, November 15-16, 2012.

Ledonio CGT, Polly DW, Brearley AM, Crawford, AH, Sucato DJ, Carreon LY, Larson AN, Stevenson D, Vitale MG, Moertel CL. *A Multicenter Inter-Observer Reliability Study of*

Radiographic Characteristics of Dystrophic Scoliosis in NF1. American Academy of Orthopaedic Surgeons (AAOS) Annual Meeting. New Orleans, LA. March 11-15, 2014.

Polly DW, Ledonio CGT, Ward K, Moertel CL, Chettier R, Nelson L, Crawford AH, Ogilvie JW. *Genetic Evaluation for the Scoliosis Gene(s) in Patients with Neurofibromatosis Type 1*. 51st Annual Meeting of the Scoliosis Research Society. Prague, Czech Republic. September 21-24, 2016. (*pending*)

Posters

Ledonio CGT, Polly DW, Brearley AM, Crawford, AH, Sucato DJ, Carreon LY, Larson AN, Stevenson D, Vitale MG, Moertel CL. *Neurofibromatosis Type I with Dystrophic Scoliosis: A Multicenter Inter-Observer Reliability Study of Radiographic Characteristics*. 19th International Meeting on Advanced Spine Techniques (IMAST); 2012, July 18-21; Istanbul, TURKEY: IMAST; 2012.

- Same poster presented at the Global Spine Congress, April 4-6, 2013. Hong Kong.

Ledonio CGT, Polly DW, Brearley AM, Larson AN, Sucato DJ, Crawford AH, Carreon LY, Stevenson D, Vitale MG, Moertel CL. *Neurofibromatosis Type I and Scoliosis: A Multicenter Study to Determine Radiographic Predictors of Dystrophic Scoliosi*s. 19th International Meeting on Advanced Spine Techniques (IMAST). Istanbul, TURKEY. July 18-21, 2012.

Ledonio CGT, Polly DW, Brearley AM, Larson AN, Sucato DJ, Carreon LY, Crawford AH, Stevenson D, Vitale MG, Moertel CL. *Neurofibromatosis Type 1 and Dystrophic Scoliosis: A Multicenter Study of Accuracy of Surgeons' Radiographic Assessment.* 19th International Meeting on Advanced Spine Techniques (IMAST). Istanbul, TURKEY. July 18-21, 2012.

- Same poster presented at:
  - Global Spine Congress, April 4-6, 2013. Hong Kong.
  - North American Spine Society ***(***NASS***)*** 28th Annual Meeting. October 9-12, 2013. New Orleans, Louisiana.

Manuscripts

Two manuscripts are in process:

- A manuscript covering the genetic segment (Aim 2) of this study will be submitted to a prominent orthopaedic journal in September 2016, prior to the podium presentation of the same at the September SRS meeting in Prague (see Presentations above).
- A manuscript covering the radiographic study (Aim 1) is being drafted; we plan to submit that to a spine-related journal in September 2016

Publications

There are currently no peer-reviewed or other journal publications related to this work.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
No change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to report

What other organizations were involved as partners?
Nothing to report

SPECIAL REPORTING REQUIREMENTS
None

Appendix A.

Table A1. The proportion of x-ray sets rated as having specific dystrophic radiographic characteristics

X-ray characteristic	Percent of 610 ratings that identified characteristic (all 5 surgeons, %)	Deemed dystrophic %	Deemed non-dystrophic %
Vertebral wedging	61.5	90.6	18.6
Vertebral rotation	61.2	89.3	19.8
Short, sharp angular curve	52.5	84.3	5.7
Rib penciling	42.8	63.1	13.0
Vertebral scalloping	40.7	57.9	15.4
Widened interpedicular distance	36.1	54.8	8.5
Atypical curve location	22.3	35.0	3.6
Spindling of TPs	15.1	23.4	2.8

* 5 surgeons*122 x-ray sets=610 ratings. 83/122 patients (68%) had pre-enrollment diagnosis of dystrophic NF1 scoliosis
TPs: transverse processes

Appendix B.

Table B1. The frequency at which specific dystrophic characteristics were observed in radiographs of NF1 patients with dystrophic scoliosis

Variable	**Rated as having characteristic out of 610 ratings** (%)	**Proportion with characteristic among truly dystrophic** (sensitivity %)	**Proportion with characteristic among truly non-dystrophic** (%)
Vertebral wedging	61.5	75.9	30.8
Vertebral rotation	61.2	76.1	29.2
Sharp angular curve	52.5	65.3	25.1
Rib penciling	42.8	54.4	18.0
Vertebral scalloping	40.7	46.8	27.7
Widened IP distance	36.1	43.9	19.5
Atypical curve location	22.3	29.6	6.7
Spindling of TPs	15.1	18.3	8.2

IP: interpedicular; TP: transverse processes

Appendix C.

Table C1. Frequency of correct dystrophic versus non-dystrophic classification by 5-surgeon panel based on single set of spinal x-rays for 122 NF1 patients with scoliosis

Number of surgeons who labeled x-rays* dystrophic	Patients with dystrophic diagnosis before enrollment n (%)	Patients without dystrophic diagnosis before enrollment n (%)	Total patients n
0	8 (9.6)	22 (56.4)	30
1	4 (4.8)	2 (5.1)	6
2	6 (7.2)	5 (12.8)	11
3	8 (9.6)	3 (7.7)	11
4	15 (18.1)	3 (7.7)	18
5	42 (50.6)	4 (10.3)	46
Total	83	39	122

*PA and lateral full spine x-rays per patient

Appendix D. ScoliScore™ Genetic Testing Results of NF1 Patients: Identification of 53 Single-Nucleotide Polymorphism (SNP) Markers[6] Associated with Scoliosis Progression in Adolescent Idiopathic Scoliosis

Fake ID	Scoli Score™	Actual diagnosis
1	23	non dystrophic
2	120	dystrophic
3	7	non dystrophic
4	3	non dystrophic
5	82	dystrophic
6	17	dystrophic
7	80	dystrophic
8	55	dystrophic
9	84	non dystrophic
10	16	non dystrophic
11	104	dystrophic
12	48	non dystrophic
13	13	non dystrophic
14	12	non dystrophic
15	2	dystrophic
16	25	dystrophic
17	43	dystrophic
18	65	dystrophic
19	12	dystrophic
20	13	non dystrophic
21	72	dystrophic
22	17	dystrophic
23	105	dystrophic
24	26	non dystrophic
25	35	dystrophic
26	43	non dystrophic
27	53	dystrophic
28	70	non dystrophic
29	7	non dystrophic
30	64	non dystrophic
31	12	non dystrophic
32	26	non dystrophic
33	3	non dystrophic
34	28	non dystrophic
35	27	dystrophic
36	133	non dystrophic
37	4	dystrophic
38	23	non dystrophic
39	24	dystrophic
40	11	dystrophic
41	21	dystrophic
42	74	dystrophic
43	57	dystrophic
44	6	non dystrophic
45	14	non dystrophic
46	5	non dystrophic
47	19	dystrophic
48	23	dystrophic
49	36	dystrophic
50	78	non dystrophic
51	39	non dystrophic
52	8	non dystrophic
53	19	dystrophic
54	3	non dystrophic
55	11	dystrophic
56	8	non dystrophic
57	61	dystrophic

Appendix E. Predicted probabilities of Dystrophic Scoliosis from Logistic Regression, based on ScoliScore™ Genetic Test (Single Predictor) in NF1 Patients

		95% CI bounds	
ScoliScore	Predicted probability	Lower	Upper
1	0.38	0.22	0.58
2	0.38	0.22	0.58
3	0.39	0.22	0.58
4	0.39	0.23	0.58
5	0.39	0.23	0.58
6	0.40	0.24	0.58
7	0.40	0.24	0.58
8	0.40	0.25	0.58
9	0.41	0.25	0.58
10	0.41	0.26	0.58
11	0.42	0.27	0.58
12	0.42	0.27	0.58
13	0.42	0.28	0.59
14	0.43	0.28	0.59
15	0.43	0.29	0.59
16	0.43	0.29	0.59
17	0.44	0.30	0.59
18	0.44	0.30	0.59
19	0.44	0.31	0.59
20	0.45	0.31	0.60
21	0.45	0.32	0.60
22	0.46	0.32	0.60
23	0.46	0.32	0.60
24	0.46	0.33	0.60
25	0.47	0.33	0.61
26	0.47	0.34	0.61
27	0.48	0.34	0.61
28	0.48	0.35	0.61
29	0.48	0.35	0.62
30	0.49	0.36	0.62
31	0.49	0.36	0.62
32	0.49	0.36	0.63
33	0.50	0.37	0.63
34	0.50	0.37	0.63
35	0.51	0.37	0.64
36	0.51	0.38	0.64
37	0.51	0.38	0.64
38	0.52	0.38	0.65

39	0.52	0.39	0.65
40	0.52	0.39	0.65
41	0.53	0.39	0.66
42	0.53	0.40	0.66
43	0.54	0.40	0.67
44	0.54	0.40	0.67
45	0.54	0.40	0.68
46	0.55	0.41	0.68
47	0.55	0.41	0.69
48	0.55	0.41	0.69
49	0.56	0.41	0.70
50	0.56	0.41	0.70
51	0.57	0.42	0.71
52	0.57	0.42	0.71
53	0.57	0.42	0.72
54	0.58	0.42	0.72
55	0.58	0.42	0.73
56	0.58	0.42	0.73
57	0.59	0.42	0.74
58	0.59	0.42	0.74
59	0.60	0.43	0.75
60	0.60	0.43	0.75
61	0.60	0.43	0.76
62	0.61	0.43	0.76
63	0.61	0.43	0.77
64	0.61	0.43	0.77
65	0.62	0.43	0.77
66	0.62	0.43	0.78
67	0.62	0.43	0.78
68	0.63	0.43	0.79
69	0.63	0.43	0.79
70	0.63	0.43	0.80
71	0.64	0.43	0.80
72	0.64	0.43	0.81
73	0.65	0.43	0.81
74	0.65	0.43	0.82
75	0.65	0.43	0.82
76	0.66	0.43	0.83
77	0.66	0.43	0.83
78	0.66	0.43	0.83
79	0.67	0.44	0.84
80	0.67	0.44	0.84
81	0.67	0.44	0.85

82	0.68	0.44	0.85
83	0.68	0.44	0.85
84	0.68	0.44	0.86
85	0.69	0.44	0.86
86	0.69	0.44	0.86
87	0.69	0.44	0.87
88	0.70	0.44	0.87
89	0.70	0.44	0.87
90	0.70	0.44	0.88
91	0.71	0.44	0.88
92	0.71	0.43	0.88
93	0.71	0.43	0.89
94	0.71	0.43	0.89
95	0.72	0.43	0.89
96	0.72	0.43	0.90
97	0.72	0.43	0.90
98	0.73	0.43	0.90
99	0.73	0.43	0.90
100	0.73	0.43	0.91
101	0.74	0.43	0.91
102	0.74	0.43	0.91
103	0.74	0.43	0.92
104	0.74	0.43	0.92
105	0.75	0.43	0.92
106	0.75	0.43	0.92
107	0.75	0.43	0.92
108	0.76	0.43	0.93
109	0.76	0.43	0.93
110	0.76	0.43	0.93
111	0.76	0.43	0.93
112	0.77	0.43	0.93
113	0.77	0.43	0.94
114	0.77	0.43	0.94
115	0.77	0.43	0.94
116	0.78	0.43	0.94
117	0.78	0.43	0.94
118	0.78	0.43	0.95
119	0.79	0.43	0.95
120	0.79	0.43	0.95
121	0.79	0.43	0.95
122	0.79	0.43	0.95
123	0.80	0.43	0.95
124	0.80	0.43	0.95

125	0.80	0.43	0.96
126	0.80	0.43	0.96
127	0.81	0.42	0.96
128	0.81	0.42	0.96
129	0.81	0.42	0.96
130	0.81	0.42	0.96
131	0.81	0.42	0.96
132	0.82	0.42	0.96
133	0.82	0.42	0.97
134	0.82	0.42	0.97
135	0.82	0.42	0.97
136	0.83	0.42	0.97
137	0.83	0.42	0.97
138	0.83	0.42	0.97
139	0.83	0.42	0.97
140	0.83	0.42	0.97
141	0.84	0.42	0.97
142	0.84	0.42	0.97
143	0.84	0.42	0.97
144	0.84	0.42	0.98
145	0.84	0.42	0.98
146	0.85	0.42	0.98
147	0.85	0.42	0.98
148	0.85	0.42	0.98
149	0.85	0.41	0.98
150	0.85	0.41	0.98
151	0.86	0.41	0.98
152	0.86	0.41	0.98
153	0.86	0.41	0.98
154	0.86	0.41	0.98
155	0.86	0.41	0.98
156	0.87	0.41	0.98
157	0.87	0.41	0.98
158	0.87	0.41	0.98
159	0.87	0.41	0.98
160	0.87	0.41	0.99
161	0.87	0.41	0.99
162	0.88	0.41	0.99
163	0.88	0.41	0.99
164	0.88	0.41	0.99
165	0.88	0.41	0.99
166	0.88	0.41	0.99
167	0.88	0.41	0.99

168	0.89	0.41	0.99
169	0.89	0.41	0.99
170	0.89	0.40	0.99
171	0.89	0.40	0.99
172	0.89	0.40	0.99
173	0.89	0.40	0.99
174	0.89	0.40	0.99
175	0.90	0.40	0.99
176	0.90	0.40	0.99
177	0.90	0.40	0.99
178	0.90	0.40	0.99
179	0.90	0.40	0.99
180	0.90	0.40	0.99
181	0.90	0.40	0.99
182	0.90	0.40	0.99
183	0.91	0.40	0.99
184	0.91	0.40	0.99
185	0.91	0.40	0.99
186	0.91	0.40	0.99
187	0.91	0.40	0.99
188	0.91	0.40	0.99
189	0.91	0.39	0.99
190	0.91	0.39	0.99
191	0.92	0.39	0.99
192	0.92	0.39	0.99
193	0.92	0.39	0.99
194	0.92	0.39	1.00
195	0.92	0.39	1.00
196	0.92	0.39	1.00
197	0.92	0.39	1.00
198	0.92	0.39	1.00
199	0.92	0.39	1.00
200	0.93	0.39	1.00

Appendix F. Abstract Accepted for Presentation at the 2016 Scoliosis Research Society Meeting

Genetic Evaluation for the Scoliosis Gene(s) in Patients with Neurofibromatosis Type 1

Drs. Polly, Ledonio, Ward, Moertel, Chettier, Nelson, Crawford, Ogilvie

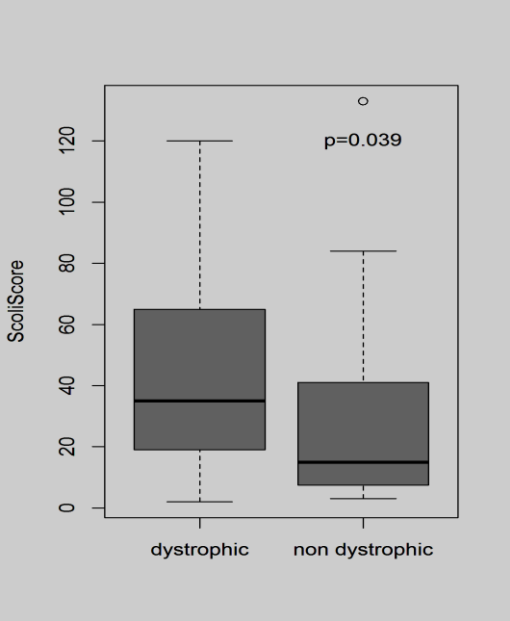
Summary:
Predictive single-nucleotide polymorphism markers for Adolescent Idiopathic Scoliosis (AIS) represented by a numerical score (ScoliScore™) are statistically associated with dystrophic scoliosis in this cohort of Neurofibromatosis type 1 (NF1) patients. There is a higher probability of a dystrophic diagnosis with higher ScoliScore. Larger sample size is needed to clinically correlate these intriguing results.

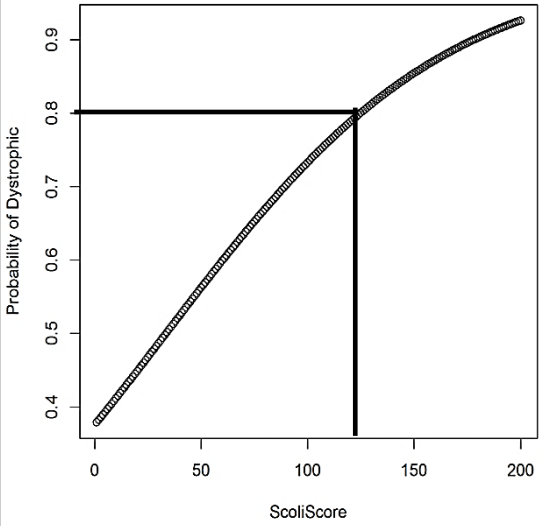
Introduction:
Scoliosis is a common skeletal manifestation in NF1. Dystrophic scoliosis can be rapidly progressive and morbid. Early intervention is recommended. Tools for early detection of dystrophic scoliosis have not been developed. The goal of this study is to evaluate if genetic markers associated with curve progression in AIS patients are predictive of dystrophic or non-dystrophic scoliosis in patients with NF1.

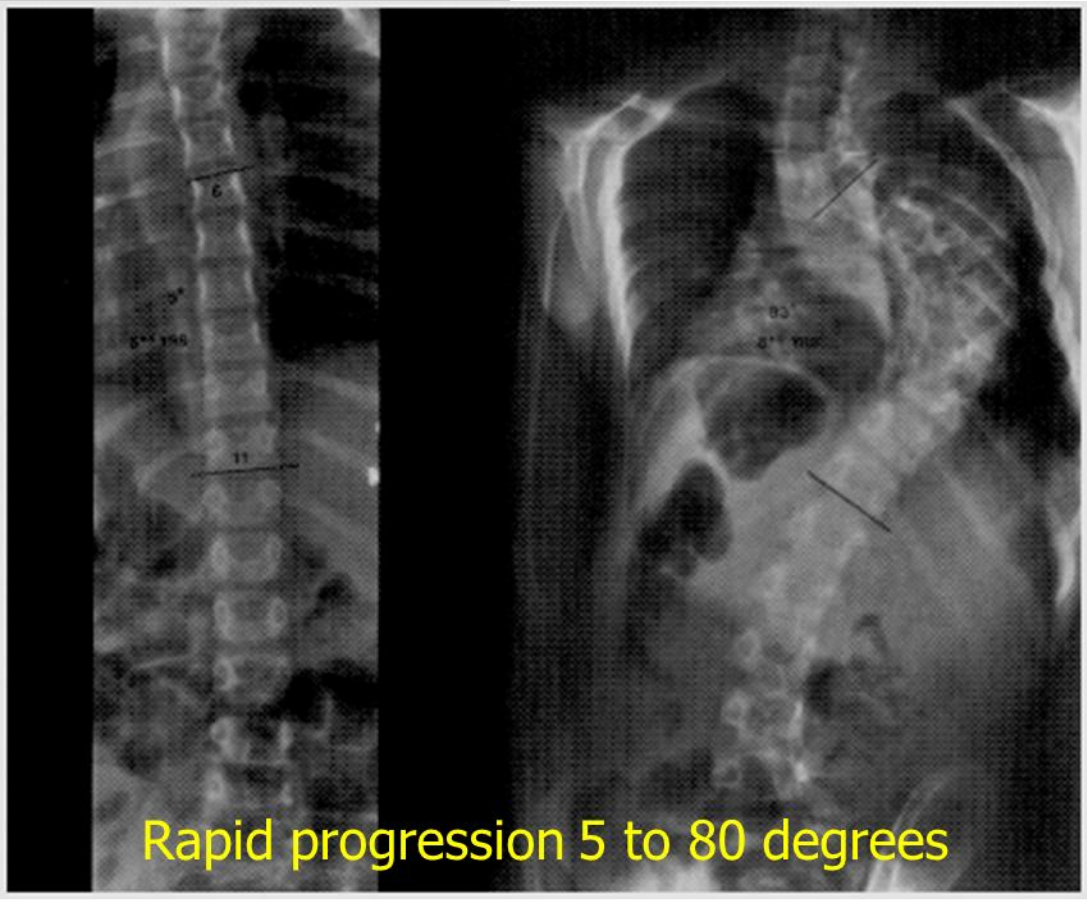
Methods:
NF1 patients with and without dystrophic scoliosis were recruited nationally from medical centers and individual solicitation. Cheek swabs were sent for genotyping with 53 single-nucleotide polymorphism (SNP) markers associated with curve progression in AIS. The genetic test results are represented as a numerical score for curve progression (ScoliScore). Scores were compared between dystrophic and non-dystrophic scoliosis using Mann-Whitney test. The logistic regression modeled the association between ScoliScore and probability of being dystrophic.

Results:
57 NF1 patients with clinical and radiographically-confirmed scoliosis were included: 29 dystrophic, 28 non-dystrophic. Average age was 22 years. ScoliScores were significantly higher among dystrophic than non-dystrophic (median 35 vs. 15, $p < 0.05$). Regression analysis showed that risk scores were able to predict the dystrophic scoliosis in NF1 patients. A Scoliscore >123 or greater yielded a likelihood of dystrophic scoliosis of > 80%.

Conclusion:
Within this small NF1 clinical cohort, there is a strong positive association between high genetic test scores (Scoliscore) and dystrophic scoliosis. These intriguing preliminary results merit further investigation. If adequately substantiated in a larger sample this would allow early detection and intervention of dystrophic modulation.


p=0.039
ScoliScore
0
20
40
60
80
100
120
dystrophic
non dystrophic


Probability of Dystrophic
0.4
0.5
0.6
0.7
0.8
0.9
0
50
100
150
200
ScoliScore


Rapid progression 5 to 80 degrees

Appendix G. List of Individuals Funded Under This Grant

Faculty:
David W. Polly, MD
Christopher Moertel, MD

Staff:
Ann Brearley, PhD
Charles Ledonio, MD
Sharon Yson, MD
Barbara J. Rogers
Ivana Ninkovic

Consultants:
James Ogilvie, MD
Kenneth Ward, MD

Travel expenses were paid for three of five surgeons from out of state who participated in the radiographic rating panel for Aim 1 (see Appendix H).

Appendix H. Five Surgeons Who Reviewed Radiographs for Aim 1

Leah Y. Carreon MD, MSc
Clinical Research Director
Norton Leatherman Spine Center
Louisville, KY

Alvin H. Crawford, MD, FACS
Professor Emeritus of Orthopaedic Surgery
Cincinnati Children's Hospital,
Cincinnati, OH

A. Noelle Larson, MD
Assistant Professor
Orthopedic Surgery
Mayo Clinic
Rochester, MN

David W. Polly, MD
James W. Ogilvie Professor and Chief of Spine Surgery
Katherine Mills Davis endowed Chair
Department of Orthopaedic Surgery
Professor of Neurosurgery
University of Minnesota
President, Scoliosis Research Society 2015-2016

Daniel J. Sucato, MD MS
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